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Antiphosphatidylserine/prothrombin antibodies as biomarkers to identify severe primary antiphospholipid syndrome

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Abstract

Background: Anti-phosphatidylserine/prothrombin (aPS/PT) antibodies have begun to be considered potentional biomarkers for antiphospholipid syndrome (APS). This cohort study investigate the role of aPS/PT antibodies as a risk factor for severe APS by evaluating the association between those antibodies and clinical/laboratory profiles of APS.

Methods: Plasma/serum samples from 197 APS patients, 100 healthy subjects and 106 patients with autoimmune diseases were collected. IgG/IgM aPS/PT antibodies were assayed using commercial ELISA kit.

Results: Prevalences of IgG and IgM aPS/PT ($p < 0.0001$ and $p = 0.0009$, respectively) and their titres ($p < 0.0001$ and $p = 0.0002$, respectively) were significantly higher in thrombosis/pregnancy group with respect to pregnancy morbidity alone. Prevalences of IgG and IgM aPS/PT ($p < 0.0001$ and $p = 0.0004$, respectively) and their mean levels ($p = 0.0001$ for both) were significantly higher in the prematurity linked to life-threatening obstetric complications group with respect to miscarriage group. There was a significant relationship between IgG and IgM aPS/PT ($p = 0.001$ and $p = 0.0002$) and their mean levels were higher ($p = 0.0004$ and $p = 0.0002$, respectively) in the thrombotic microangiopathy group, considered a milestone manifestation of catastrophic APS. The relationship between IgG and IgM aPS/PT was significant and mean levels were higher in triple positive antiphospholipid antibody patients than in double and single positivity ones ($p < 0.0001$ for all).

Conclusions: APS/PT antibodies were associated to severe thrombosis, severe pregnancy complications inducing prematurity, and vascular microangiopathy, all generally associated to high risk APS forms requiring strong therapy.

Keywords: antiphospholipid syndrome; anti-phosphatidylserine/prothrombin antibodies; lupus anticoagulant; pregnancy complications; thrombotic microangiopathy; thrombosis.

Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thrombosis, and/or pregnancy morbidity and the presence in the blood of persistent antiphospholipid antibodies (aPL). The current laboratory classification criteria include confirmed, medium-high levels of IgG/IgM anti-β_2 glycoprotein I (anti-β2GPI), and/or IgG/IgM anticardiolipin (aCL) antibodies, and/or lupus anticoagulant (LAC) activity [1]. In the last decades anti-phosphatidylserine/prothrombin (aPS/PT) antibodies are emerging as biomarkers for APS [2]. Despite, the wide variability in the patients selection and in the study design, most of the studies addressing the clinical significance of aPS/PT antibodies have shown an association with APS manifestations [3, 4]. In particular, a systematic review of 7000 patients, suggested that aPS/PT antibodies represent a strong risk factor for thrombosis irrespective of the site and type [5]. Furthermore, a correlation between aPS/PT antibodies and LAC have been reported, so raising the hypothesis that the aPS/PT test may be a confirmatory tool for LAC activity [6–8]. These data were confirmed by a study of our group showing IgG/IgM aPS/PT antibodies as an independent risk factors for the presence of LAC or thrombosis in patients with primary APS [9]. Moreover, a significant prevalence of aPS/PT antibodies ($p = 0.043$) was found in patients with clinical and without laboratory criteria for APS classification [10]. Recently, by using a global APS score, was found that positivity for aPS/PT antibodies is a significant variable when assessing the

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risk for developing thrombosis in primary APS thus suggesting that the addition of these antibodies can help in predicting APS-related clinical manifestations [11]. To go a step further we designed this study in order to explore the role of aPS/PT antibodies as a risk factor of severe APS. Therefore, the association of aPS/PT antibodies with different clinical and laboratory profiles of primary APS patients has been evaluated.

Materials and methods

Study design and participants

This is a 10-year retrospective, cohort study. Plasma and serum samples were collected prospectively from June 2005 to May 2015 from patients attending Rheumatology Unit of the University of Padova Medical Centre. All plasma and sera samples were stored at – 80 °C until tested. Patients clinical and laboratory records were collected in a dedicated database. All charts recorded in the database were carefully reviewed by two authors independently (AH, EM). All variables reported were collected on all of the subjects included in this study.

One-hundred ninety-seven primary APS patients, fulfilling the laboratory and clinical criteria for APS outlined in the Sydney Consensus Statement [1] were the study group. Patients with definite connective tissue diseases or lost to follow-up were excluded from the study. Demographic, clinical and laboratory features of primary APS patients are reported in Table 1. Thrombosis was arterial in 67 (47.5%) cases, venous in 32 (22.7%) and arterial plus venous in 42 (29.8%). Patients with pregnancy morbidity had late foetal loss in 48 (55.8%) cases, premature birth in 8 (9.3%), early abortions in 20 (23.3%), both late foetal loss and premature birth in 7 (8.1%), both late foetal loss and early abortion in 2 (2.3%) and both late foetal loss, premature birth and early abortion in one (1.2%). One hundred healthy blood donors age- and sex-matched with the primary APS patients and 106 subjects with other autoimmune diseases (99 females and 7 males, mean age = 44 years ± 14 SD, range between 19 and 74) were the control group.

The institutional review board for observational studies and the Audit Committee of the University-Hospital of Padova approved the study design. The study was carried out in accordance with the principles outlined in the Declaration of Helsinki. Once the patients were informed, they were asked to sign informed consent forms.

Laboratory assays

IgG/IgM aPS/PT antibodies were assayed using a commercial, validated [12] ELISA kit, kindly provided by the INOVA Diagnostics, Inc. (San Diego, CA, USA) following the manufacturer's instructions. The cut-off values, calculated on 100 healthy blood donors, were 61.4 and 56.3 U/mL, for IgG and IgM isotypes, respectively.

IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies were assayed using "home-made" ELISA assays, following the European Forum on aPL recommendations [13, 14], as previously described [15]. Briefly, for IgG/IgM aCL, a series of sera traceable to the Harris standard sera [16] have been used as calibration curves. Results are expressed in GPL and MPL. For IgG/IgM anti-β2GPI we used a home-made standard curve obtained from a pool of positive samples calibrated to Koike's monoclonal antibodies, HCAL for IgG and EY2C9 for IgM anti-β2GPI antibodies [17]. Results are expressed in arbitrary U/mL. Cut-off values for medium-high levels of IgG/IgM aCL and anti-β2GPI antibodies were calculated as > the 99th percentile using sera from 100 healthy blood donors. Calibrators, patient and control samples were tested in duplicate. In accordance with the Clinical and Laboratory Standards Institute protocol EP15-A2 [18] the intra- and inter-assay coefficients of variation (CV) were calculated showing values < 10% for all the tests. In particular, the intra-assay CV of IgG and IgM aPS/PT antibodies were 5.3% and 3.3%, respectively, while they inter-assay CV were 6.0% and 5.8%, respectively. The intra-assay CV of IgG aCL, IgM aCL, IgG aβ2GPI and IgM aβ2GPI were 5.4%, 7.9%, 8.7% and 5.2%, respectively, while they inter-assay CV were 4.7%, 5.2%, 8.2% and 3.3%, respectively.

Table 1: Demographic, clinical and laboratory characteristics of the study cohort.

	Total patients n = 197	Thrombosis and pregnancy morbidity n = 30	Thrombosis alone n = 111	Pregnancy morbidity n = 56
Age, mean ± SD	44 ± 12	40 ± 7.8	48 ± 13.6	39 ± 5.8
Females n (%)	161 (81.7)	30 (100)	75 (67.6)	56 (100)
Males n (%)	36 (18.3)	0 (0)	36 (32.4)	0 (0)
Laboratory features				
IgG aCL n (%)	136 (69)	23 (76.7)	90 (81.1)	23 (41.1)
IgM aCL n (%)	79 (40.1)	4 (13.3)	55 (49.5)	20 (35.7)
IgG anti-β2GPI n (%)	141 (71.6)	25 (83.3)	91 (81.9)	25 (44.6)
IgM anti-β2GPI n (%)	88 (44.7)	6 (20)	59 (53.2)	23 (41.1)
LAC n (%)	113 (57.7)	27 (90)	81 (73.6)	5 (8.9)
IgG aPS/PT n (%)	59 (29.9)	18 (60)	37 (33.3)	4 (7.1)
IgM aPS/PT n (%)	95 (48.2)	19 (63.3)	62 (55.9)	14 (25)

IgG, immunoglobulin G; IgM, immunglobulin M; aCL, anticardiolipin antibodies; anti-β2GPI, anti-β2 glycoprotein I antibodies; LAC, lupus anticoagulant; aPS/PT, antiphosphatidylserine/prothrombin antibodies.

LAC was detected following internationally accepted recommendations [19] using dilute Russell Viper Venom and dilute Activated Partial Thromboplastin Times as screening tests. Samples with a prolonged screening test not corrected by mixing with normal pooled plasma were tested for confirmation using an excess of phospholipids.

We considered IgG and/or IgM isotype as a single antibody positivity. Therefore, single aPL positivity referred to LAC or IgG/IgM aCL or IgG/IgM anti-β2GPI; double positivity referred to IgG/IgM aCL plus IgG/IgM anti-β2GPI or IgG/IgM aCL plus LAC or IgG/IgM anti-β2GPI plus LAC; triple positivity referred to IgG/IgM aCL plus IgG/IgM anti-β2GPI plus LAC.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software, version 22.0 (Chicago, IL, USA). The association between aPS/PT antibodies and clinical features of primary APS were evaluated using Fisher's exact test, the odds ratio (OR) with a 95% confidence interval (CI) was calculated. The Mann-Whitney test and Kruskall-Wallis test followed by post-hoc analysis (Dunn tests) were employed to compare mean aPS/PT antibody levels in the different clinical and laboratory subsets of primary APS. A stepwise forward conditional procedure was used for logistic regression analysis to evaluate the independent risk factors of severe primary APS. A p-value < 0.05 was considered statistically significant.

Results

Prevalences of IgG and IgM isotypes in all APS patients and in the different clinical subsets are outlined in Table 1. The sensitivity of IgG and IgM aPS/PT antibodies for APS classification was 29.9% and 48.2%, respectively, and their specificity of 97.6% and 97%, respectively. Both IgG and IgM aPS/PT frequencies were significantly higher in APS patients than in the control group, $p < 0.0001$ for both, (OR 17.2, 95% CI 6.7–43.9 and OR 31.1, 95% CI 13.2–73.3, respectively).

Association of aPS/PT antibodies with APS clinical subsets

Higher prevalences of both IgG and IgM isotypes were found in patients with thrombosis alone with respect to those with pregnancy morbidity alone (OR 6.5; 95% CI, 2.2–19.4 and OR 3.8; 95% CI, 1.9–7.7, respectively) (Figure 1A). A significantly higher prevalence of IgG aPS/PT antibodies was found in the women with both thrombosis and pregnancy morbidity with respect to those with thrombosis alone (OR 3.0; 95% CI, 1.3–6.9) or pregnancy morbidity alone (OR 19.5; 95% CI, 5.6–68.2) (Figure 1A). Both males and females have been considering in the TAPS cohort. However, the results did not shown a different outcome by comparing only females. The frequency of IgM aPS/PT antibodies was significantly higher in the patients with both thrombosis and pregnancy morbidity than in those with pregnancy morbidity alone (OR 5.2; 95% CI, 2.0–13.5), but there was no significant difference between the patients with both thrombosis and pregnancy morbidity and those with thrombosis alone (Figure 1A). As shown in Figure 1B patients with both thrombosis and pregnancy morbidity had a higher median IgG aPS/PT antibody titres 141.1 (25th–75th interquartile range 21.6–589.1) vs. 18.9 (25th–75th interquartile range 7.5–124.9) and 9.4 (25th–75th interquartile range 7–17.8), respectively in those with thrombosis alone and pregnancy morbidity alone ($p < 0.0001$). Median IgM aPS/PT antibody titres (Figure 1C) were higher in patients with both thrombosis and pregnancy morbidity 79.8 (25th–75th interquartile range 32.8–214.6) vs. 68.8 (25th–75th interquartile range 20.9–172.6) and 32.7 (25th–75th interquartile range 18.6–60.0), respectively in those with thrombosis alone and pregnancy morbidity alone ($p = 0.0002$).

Concerning the type of vascular involvement, as Figure 2A shows, IgG aPS/PT but not IgM aPS/PT antibodies had a significantly higher frequency in patients with both arterial and venous thrombosis than in those with arterial or venous thrombosis alone (OR 2.2, 95% CI 1.1–4.6). Within the vascular involvement (Figure 2B and C) nor was there any significant difference between median IgG and IgM aPS/PT antibodies titres obtained in patients with both arterial and venous thrombosis 73 (25th–75th interquartile range 8.7–607.8) and 96.5 (25th–75th^{-} interquartile range 20.6–216.3), respectively vs. those with arterial or venous thrombosis 21.8 (25th–75th interquartile 8.5–141.4) and 68.5 (25th–75th interquartile range 24.4–172.6), respectively.

As far as pregnancy morbidity was concerned, the prevalence of IgG aPS/PT antibodies was significantly higher in women with prematurity with respect to those with foetal loss (OR 5.3; 95% CI, 1.6–17.6) and those with miscarriages (OR 66.2; 95% CI, 3.4–1293.0); but, as outlined in Figure 2D, there was no significant difference between patients with foetal loss and those with miscarriages. As shown in Figure 2E, within the pregnancy morbidity median IgG aPS/PT antibody titres were significantly higher in women with prematurity 141.1 (25th–75th interquartile range 19.9–1056.0) vs. 15.3 (25th–75th interquartile range 8.7–48.9) and 7.9 (25th–75th interquartile range 5.6–14.6), respectively in those with foetal loss and miscarriages ($p = 0.0001$). IgM aPS/PT antibodies were

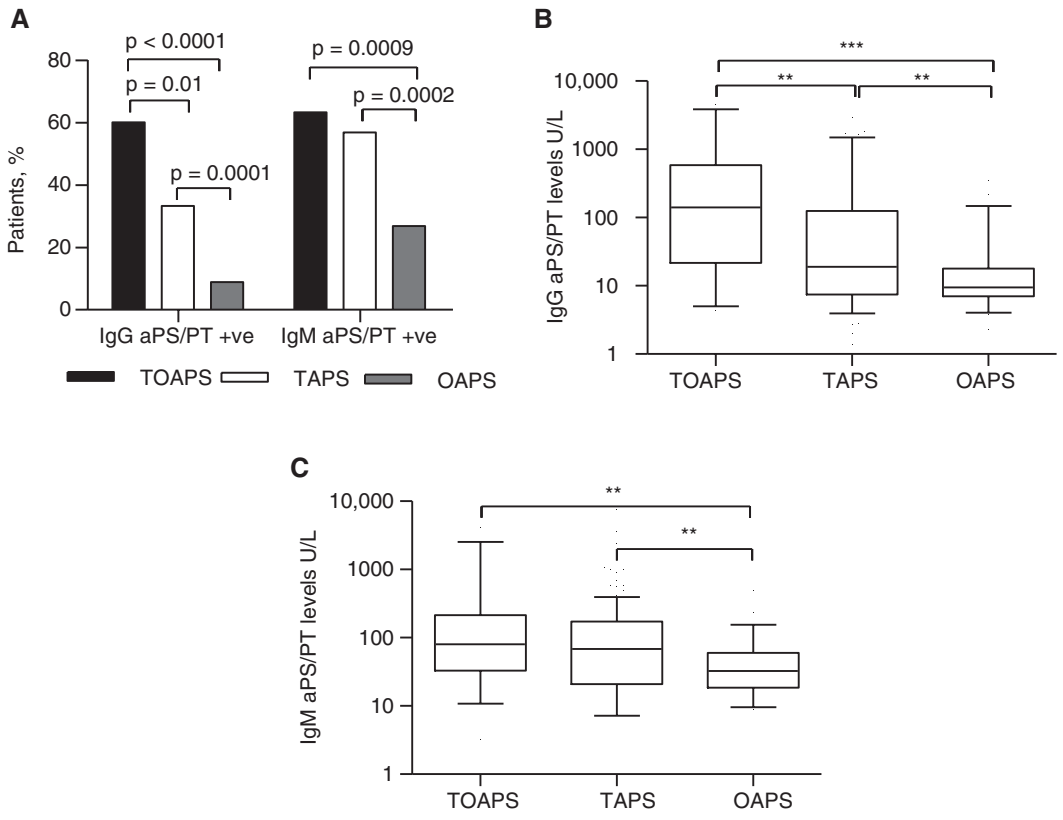

Figure 1: The association of IgG and IgM aPS/PT antibodies with the different clinical subsets of the primary antiphospholipid syndrome patients studied (A), IgG (B) and IgM (C) aPS/PT antibody levels in the different clinical subsets of the primary antiphospholipid syndrome. Data are shown as Tukey box plots, where each box represents the 25th–75th percentiles: lines inside the box represent the median. The whiskers represent the 1.5 interquartile range of the 25th and 75th quartile. TOAPS n = 30; TAPS n = 111; OAPS n = 56.aPS/PT = antiphosphatidylserine/prothrombin; TOAPS = thrombosis and pregnancy morbidity; TAPS = thrombosis alone; OAPS = pregnancy morbidity alone. *p < 0.05; **p < 0.01; ***p < 0.0001.

significantly associated with prematurity with respect to miscarriages (OR 19.8; 95% CI, 3.2–120.2) and with foetal loss with respect to miscarriages (OR 6; 95% CI, 1.3–28.7); but there was no significant difference between prematurity and foetal loss (Figure 2D). As shown in Figure 2F, median IgM aPS/PT antibody levels were significantly different in patients with prematurity 100.5 (25th–75th interquartile range 40.8–216.8) vs. 43.67 (25th–75th interquartile range 23.1–87.6) and 22.9 (25th–75th interquartile range 15.0–33.7), respectively in those with foetal loss and miscarriages (p = 0.0001).

The prevalences of IgG and IgM isotypes were both significantly higher in the patients with microangiopathy (OR 3.1; 95% CI, 1.6–6.1 and OR 3.6; 95% CI, 1.8–7.4, respectively) (Figure 3A). In addition, both median IgG and IgM antibody levels were significantly higher in patients with microangiopathy 57.8 (25th–75th interquartile range 13.3–277.7) and 108.4 (25th–75th interquartile range 35.2–329.4), respectively than in those without 13.5 (25th–75th interquartile range 7.2–50.0) and 38.6 (25th–75th interquartile range 20.1–107.6), respectively (Figure 3B and C). Microangiopathy was, moreover, an independent risk factor associated to severe primary APS (p = 0.006; OR 4.3; 95% CI, 1.5–12.1) at multivariate logistic regression analysis.

Association of APS/PT antibodies with APS laboratory subsets

As reported in Figure 4A, the frequency of IgG was significantly higher in patients with triple aPL positivity with respect to those with double or single positivity (OR 8.3; 95% CI, 3.4–20.0 and OR 5.7; 95% CI, 2.8–11.7, respectively). In addition, the prevalence of IgM adPS/PT antibodies was significantly higher in patients with triple aPL positivity with respect to those with double and single positivity (OR 22.8; 95% CI, 5.2–99.6 and OR 9.5; 95% CI, 4.0–22.3, respectively). Median IgG and IgM antibody levels (Figure 4B and C) were significantly higher in triple aPL positive patients 78.0 (25th–75th interquartile range 17.5–468.9) and 108.7

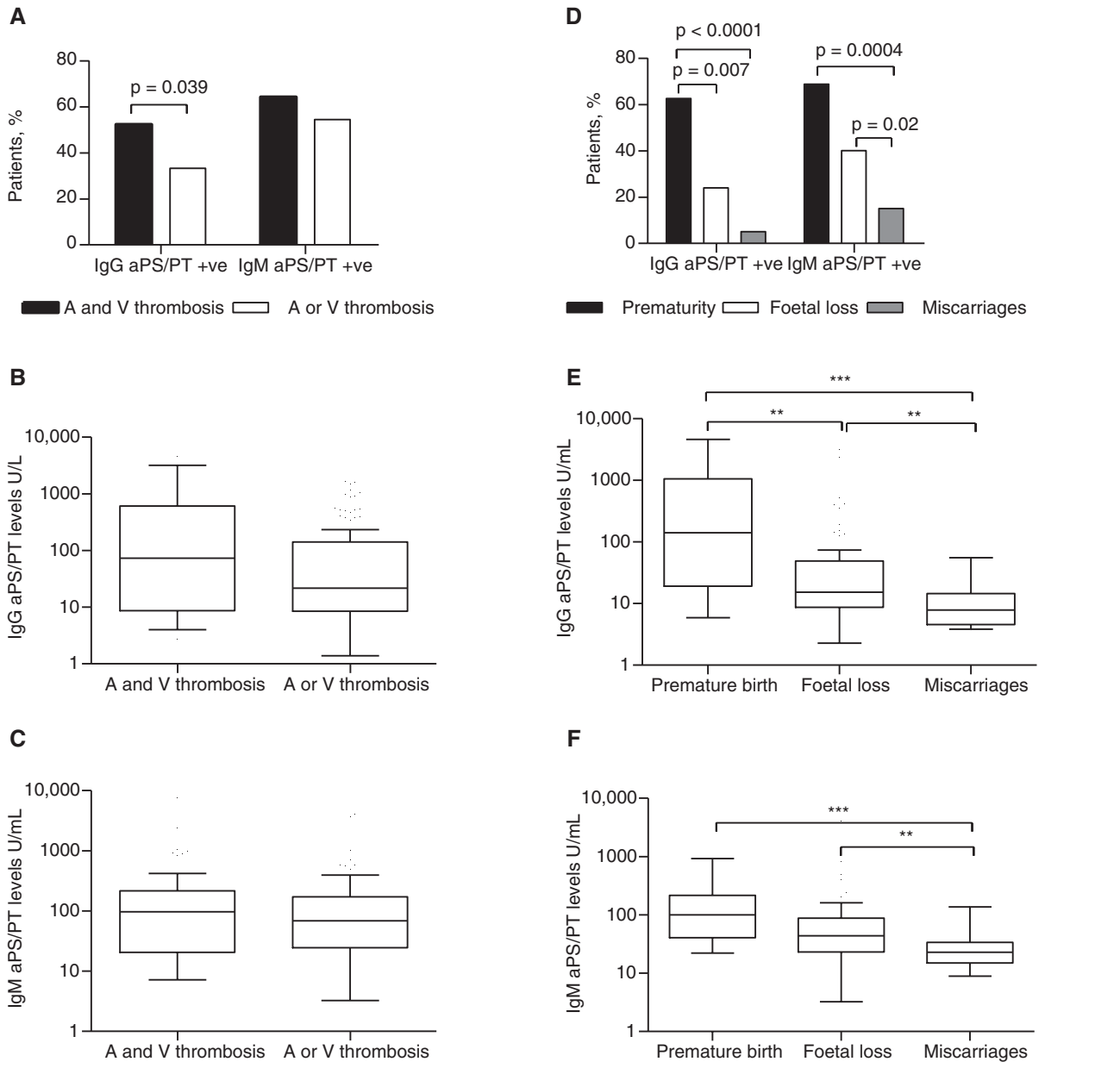

Figure 2: The association of IgG and IgM aPS/PT antibodies with the thrombosis features (A) and with the pregnancy morbidity features (D). IgG and IgM aPS/PT antibody levels in the thrombosis subsets (B, C) and in the pregnancy morbidity subsets (E, F).
Data are shown as Tukey box plots, where each box represents the 25th–75th percentiles: lines inside the box represent the median. The whiskers represent the 1.5 interquartile range of the 25th and 75th quartile. A and V thrombosis n = 42, A or V thrombosis n = 99, premature birth n = 16, foetal loss n = 50, miscarriages n = 20. aPS/PT = antiphosphatidylserine/prothrombin; A = arterial; V = venous. *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$.

(25th–75th interquartile range 45.9–243.7), respectively than in those with double 10.3 (25th–75th interquartile range 7.0–22.5) and 33.1 (25th–75th interquartile range 18.6–78.3), respectively and single positivity 8.1 (25th–75th interquartile range 5.1–14.2) and 23.3 (25th–75th interquartile range 14.9–53.2), respectively, ($p < 0.0001$ for both).

When the association between IgG and IgM aPS/PT antibodies and LAC alone or associated to either aCL or to anti-β2GPI was examined (Figure 4D), the prevalence of both aPS/PT isotypes was higher in LAC positive patients with respect to their negative counterparts (OR 25.3; 95% CI, 7.5–84.8 and (OR 9.7; 95% CI, 4.9–19.2, respectively). Furthermore, as shown in Figure 4E and F, both median IgG and IgM aPS/PT antibody titres were significantly higher in LAC positive patients 53.1 (25th–75th interquartile range 14.4–401.6) and 108.7 (25th–75th interquartile

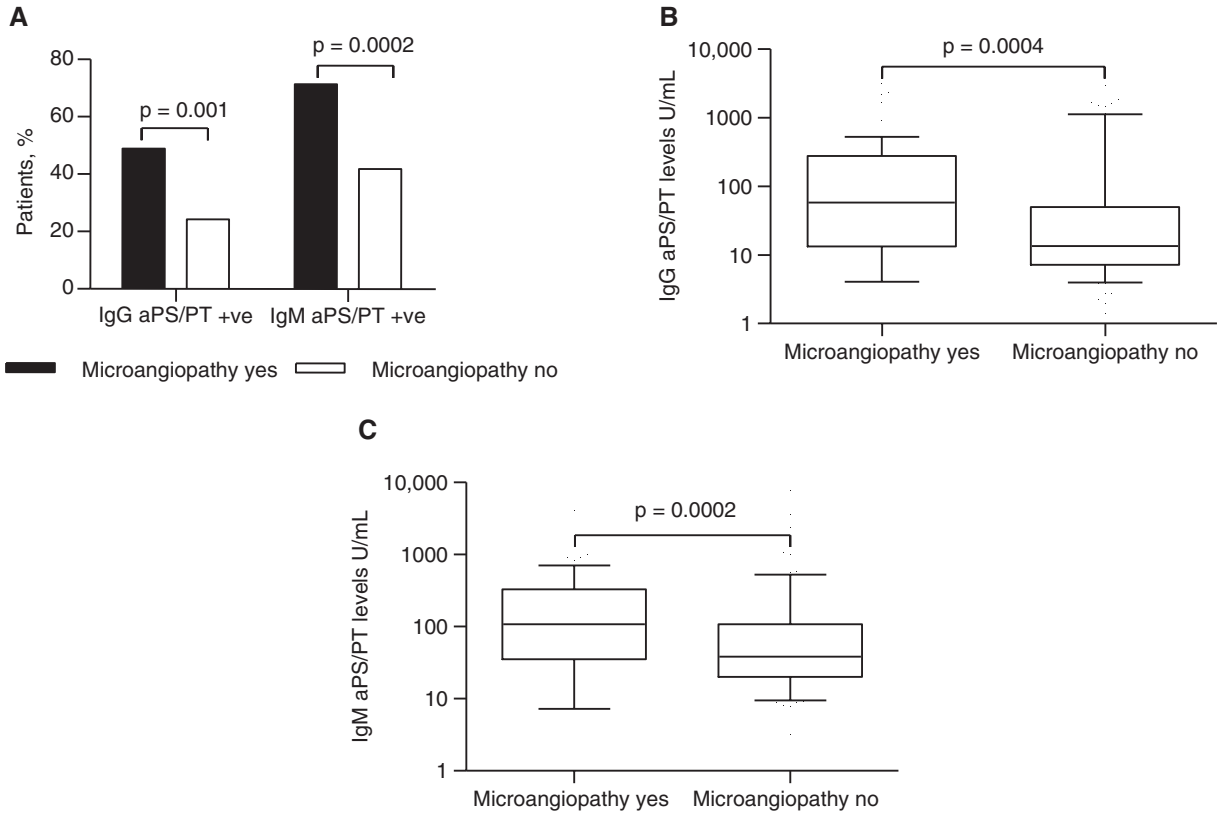

Figure 3: The association of IgG and IgM aPS/PT antibodies with the microangiopathy manifestation (A). IgG and IgM aPS/PT antibody levels in the thrombotic microangiopathy subsets (B, C).
Data are shown as Tukey box plots, where each box represents the 25th–75th percentiles: lines inside the box represent the median. The whiskers represent the 1.5 interquartile range of the 25th and 75th quartile. Microangiopathy yes n = 49, microangiopathy no n = 148. aPS/PT, antiphosphatidylserine/prothrombin.

range 43.7–241.8), respectively with respect to their negative counterparts 8.5 (25th–75th interquartile range 5.9–15.5) and 27.2 (25th–75th interquartile range 15.8–42.9).

Discussion

This study showed, in a large cohort of patients with primary APS, a significant association of aPS/PT antibodies with clinical and laboratory features of severe APS.

Indeed, a higher prevalence and a higher mean titres of both IgG and IgM of aPS/PT antibodies were found in patients with thrombosis alone than those with pregnancy morbidity alone. Moreover, IgG aPS/PT antibodies significantly prevailed and showed higher mean levels in women with both thrombosis and pregnancy morbidity than in those with thrombosis alone or pregnancy morbidity alone. These findings are in accordance with previous data [5, 9, 20–22] showing that aPS/PT antibodies are a strong risk factor for vascular thrombosis given their significant relationship with the more severe clinical subsets of APS. It is important to remember that women with both thrombosis and pregnancy morbidity are considered at high risk of pregnancy complications, a disease subset often refractory to conventional therapy [23].

The novelty of the present study is the association of IgG aPS/PT antibodies with the more severe picture of vascular thrombosis. Previous studies examining the association between aPS/PT antibodies with thrombosis type in APS patients have demonstrated a significant association with arterial or venous thrombosis, probably due to the patient selection criteria used [3, 5, 9, 20–22]. Interestingly, our findings uncovered a significant association between IgG aPS/PT antibodies and a more severe form of vascular thrombosis characterized by both arterial and venous thrombosis involvement. Moreover, the prevalence of aPS/PT antibodies was significantly higher in women with life-threatening obstetric complications such as severe preeclampsia, eclampsia or haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome which induces placental insufficiency and prematurity. The presence of

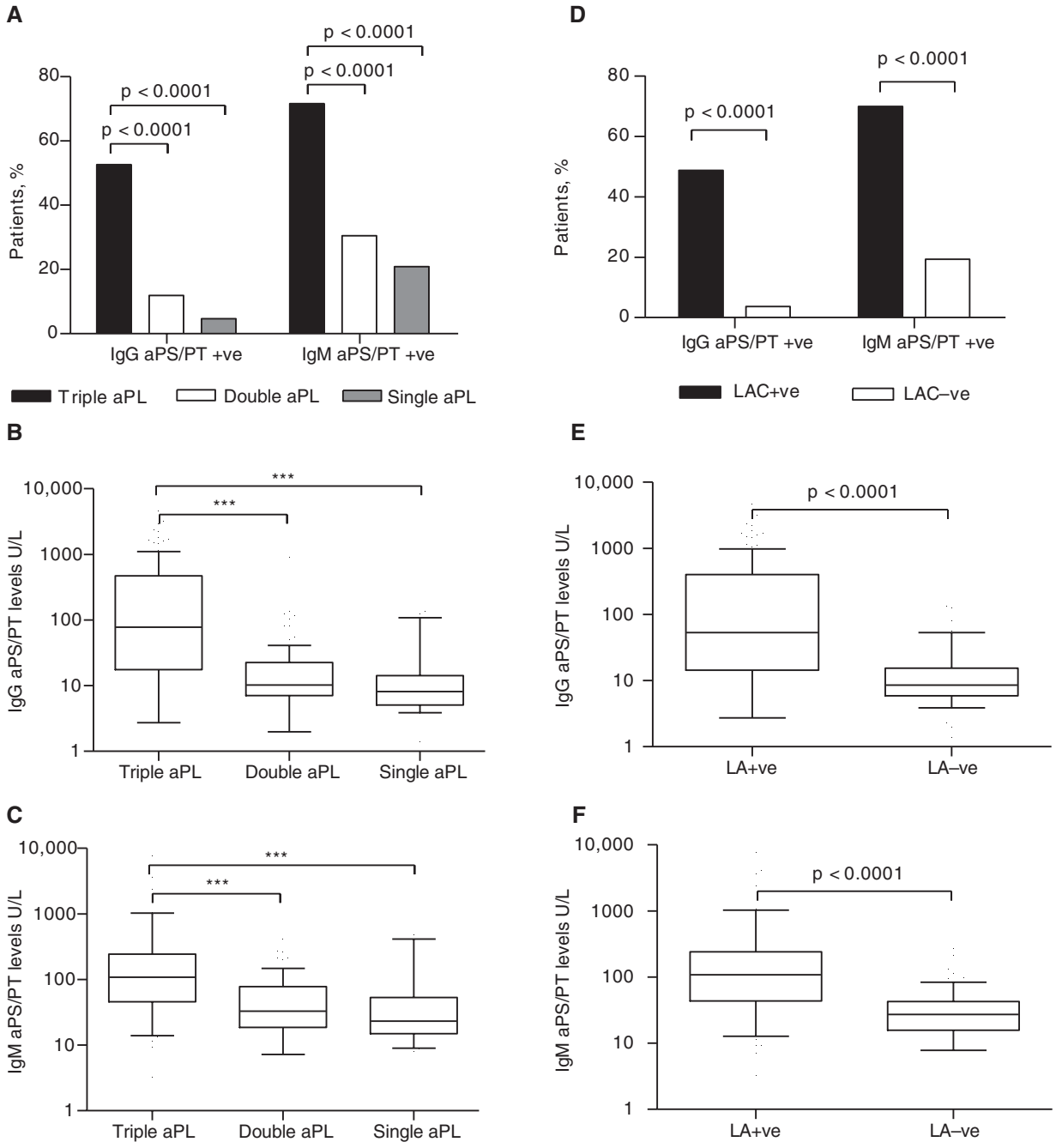

Figure 4: The association between IgG and IgM aPS/PT antibodies with conventional antiphospholipid antibody profiles (A) and with positivity or negativity to lupus anticoagulant (D). IgG and IgM aPS/PT antibody levels in the three conventional antiphospholipid antibody profiles (B and C) and in thepresence or absence of lupus anticoagulant (E and F).
Data are shown as Tukey box plots, where each box represents the 25th–75th percentiles: lines inside the box represent the median. The whiskers represent the 1.5 interquartile range of the 25th and 75th quartile. Triple aPL n = 95, double aPL n = 59, single aPL n = 43, LAC + ve n = 114, LAC – ve n = 83. aPS/PT, antiphosphatidylserine/prothrombin; aPL, antiphospholipid antibody; LAC + ve, lupus anticoagulant positive; LAC – ve, lupus anticoagulant negative. ***p < 0.0001.

these complications in an APS context could be explained at least in part by the interplay between aPL and endothelial cells leading to thrombosis and microangiopathic manifestations at the maternal-foetal interface [24, 25].

To the best of our knowledge, this is the first time that the association between aPS/PT antibodies and thrombotic microangiopathy has been examined. The results demonstrate that there is indeed a significant

correlation and higher mean IgG and IgM aPS/PT antibodies levels in this subset of patients. APS microangiopathy characterized by vascular small blood vessel occlusion of different tissues and organs is the milestone manifestation of catastrophic APS, a rare life-threatening form of APS. Microangiopathy was, in fact, the only independent risk factor of severity in our cohort of primary APS patients.

According to revised classification criteria, APS patients are allocated to categories on the basis of positivity to more than one test (category I) or to a single test (category II) [1]. Association with clinical events is clear in category I patients, while it is lacking or weak in patients belonging to category II [26]. Triple aPL positivity, referring to IgG/IgM aCL plus IgG/IgM anti-β2GPI plus LAC, is considered the most predictive profile for severe clinical APS manifestations [27, 28]. A higher prevalence of both IgG and IgM aPS/PT antibodies was found in patients with triple aPL with respect to the ones with double or single positivity. Moreover, their mean levels were significantly higher in triple aPL than in double and single aPL positivity.

When we evaluate the association of aPS/PT antibodies with LAC alone and/or associated with aCL or anti-β2GPI we observed a significant association of both IgG and IgM aPS/PT antibodies with LAC alone or associated. LAC, is the most important laboratory criterion for APS classification because it is considered a strong risk factor for thrombosis and foetal loss [29]. Recently has been reported a higher rate of LAC in patients with stroke, myocardial infarction, deep venous thrombosis and both early and late pregnancy morbidity [30]. Moreover, LAC positivity has emerged in the majority of studies as the strongest predictor of pregnancy morbidity [23, 31], and it is, in fact, significantly associated with poor infant outcome [32]. Finally, it has been found to be an independent risk factor for thrombosis in aPL carriers [33].

Several studies have shown that phosphatidylserine interacts with β2GPI present in patients' sera [4]. In view of this finding, the ability of aPS/PT assays to detect antiprothrombin antibodies could be debatable, leading the question if it is actually identifying anti-β2GPI antibodies by allowing β2GPI interaction with immobilized phosphatidylserine. These antibodies might then interfere with aPS/PT evaluation. It is important to remember in any case that since β2GPI is a cationic molecule incapable of binding phosphatidylserine in the presence of calcium ions, that possibility must be excluded as the commercial aPS/PT ELISA kit used by us does contain calcium ions, both in the incubation and washing buffer. Furthermore, we did not find any reactivity when affinity purified anti-β2GPI antibodies from high risk APS patients were tested in aPS/PT ELISA plates (data not shown). Therefore, the association of aPS/PT with APS patients was not due to false detection of anti-β2GPI recognizing β2GPI-PS complexes.

Conclusions

Despite the retrospective nature of the study, we can state that aPS/PT antibodies could be considered associated to a severe thrombosis, severe pregnancy complications inducing prematurity and vascular microangiopathy which are generally associated to high risk APS, so requiring a strong therapy. These findings, if confirmed by large multicenter prospective studies, might induce to consider aPS/PT antibodies as a predictive marker of severe APS.

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